Magnetic Circulating DNA Kit

Cat.No.: HYC235

Product Description

This kit is designed for purification and recovery of cell-free DNA (cfDNA) from 1–5 mL cell-free body fluids including plasma, serum and urine. It integrates room-temperature lysis and nucleic acid binding into a single step without ice incubation. There is no need for intermittent addition of binding buffer or magnetic beads, which greatly simplifies the workflow. Under high-salt conditions, cell-free DNA binds to the surface of silica-coated magnetic beads. After washing steps, purified cell-free DNA is eluted in nuclease-free water. The yield of cell-free DNA varies significantly depending on sample type, storage conditions, storage duration and individual biological differences. This kit features high extraction efficiency, excellent reproducibility, simple, rapid and streamlined operation. The purified cell-free DNA exhibits consistent and reliable quality, and is suitable for routine downstream applications such as quantitative PCR and next-generation sequencing library construction.

Components

Components	48 T
Buffer CTL	2×72 mL
Buffer CTW	60 mL
Buffer GCW2*	2×10 mL
RNase-Free Water	10 mL
Proteinase K	5 mL
Magnetic Microspheres N	2×1 mL

Storage

Stored in a dry place at room temperature (15-30℃).

Required Reagents and Equipment

- Absolute Ethanol

Instructions

Preparations

- Prior to first use, add anhydrous ethanol to Buffer GCW2 as instructed on the reagent bottle label.
- Magnetic Microspheres N must not be frozen or subjected to high-speed centrifugation, which may cause irreversible damage to the magnetic beads. Vortex Magnetic Microspheres N vigorously for 30 seconds to achieve uniform suspension before each use.
- Inspect Buffer CTL and Buffer CTW for crystals or precipitates before use. If precipitates or crystals form, incubate the buffers in a 50 °C water bath for several minutes until fully clarified.
- Process fresh samples promptly, or aliquot and store frozen at −70 °C. Repeated freeze-thaw cycles should be avoided. After thawing frozen samples, centrifuge at 2000 × g for 1 min and collect the supernatant for subsequent experiments.

Protocol

1. Add reagents into the centrifuge tube in sequence: 100 µL Proteinase K, 2 mL plasma, 3 mL Buffer CTL and 30 µL Magnetic Microspheres N. Refer to the attached table for volumes of other sample types.

   Note: Add reagents strictly in the listed order to prevent Proteinase K inactivation. Do not add Buffer CTL directly into Proteinase K solution.
2. Thoroughly mix samples and reagents, then incubate on a thermomixer with shaking at 1200 rpm for 20 min at room temperature, or on a plate shaker at 70 rpm for 20 min to maintain magnetic bead suspension and sufficient nucleic acid binding. Spin briefly to collect liquid droplets on the inner tube wall after incubation.
3. Place the tube on a magnetic rack and stand for 2 min until beads are fully captured and the solution turns clear. Invert the tube to wash residual beads off the cap, leave for another 1 min, then discard supernatant.
4. Add 750 µL Buffer CTW and vortex for 30 s to fully resuspend beads. Spin briefly to clear liquid from the tube cap.

Note: All liquid and beads from Step 4 can be transferred into a 2 mL centrifuge tube for subsequent steps. If beads remain adhered to the tube wall, add an extra 100 µL Buffer CTW for rinsing before transfer.

5. Mount the tube on the magnetic rack and incubate for 1 min. Discard supernatant gently once all beads are captured by the magnet.
6. Add 750 µL Buffer GCW2 (confirm anhydrous ethanol has been supplemented before use), vortex for 30 s, then spin briefly to remove liquid from the tube cap.
7. Fix the tube on the magnetic rack for 1 min, then carefully discard supernatant after complete bead capture.
8. Repeat Steps 6 and 7 once.
9. Leave the tube open on the magnetic rack and air-dry at room temperature for 5–10 min.
   Note: Residual ethanol inhibits downstream enzymatic reactions; ensure full ethanol evaporation. Stop drying when beads appear matte without cracking. Over-drying will lead to difficult DNA elution.
10. Remove the tube from the magnetic rack, add 50–100 µL RNase-Free Water and vortex to fully resuspend beads in elution buffer. Perform elution incubation on a thermomixer with shaking at 1600 rpm for 10 min at room temperature.
11. Place the tube on the magnetic rack for 2 min until all beads adhere to the tube wall. Transfer the eluate containing purified DNA to a new centrifuge tube with a pipette; store at −20 ± 5 °C for later use.